BARRIER MECHANISMS FOR NEUROTRANSMITTER MONOAMINES AND THEIR PRECURSORS AT THE BLOOD-BRAIN INTERPHASE

BY

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Lund 1978

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AKADEMISK AVHANDLING

som med vederbörligt tillstånd av Medicinska Fakulteten i Lund för vinnande av medicine doktorsexamen kommer att offentligen försvaras å Histologiska institutionens föreläsningssal (nya byggnaden), Lund, tisdagen den 19 december 1978 kl. 9.15 (precis)

av

JAN ERIK HARDEBO
med.lic., Mlm.

Dokumentutgivare
04T0 University of Lund

Handläggare
06T0 Department of Neurology and Histology

Författare
08T0 Jan Erik Hardebo

Dokumentnamn
04T4 Dissertation

Utgivningsdatum
06T4 December 1978

Dokumentbeteckning
04T6 LUMEDW/(MENK-1001)

Ärendebeteckning
06T6 1-140/1978

10T4

Dokumenttitel och undertitel
18T0 BARRIER MECHANISMS FOR NEUROTRANSMITTER MONOAMINES AND THEIR PRECURSORS AT THE BLOOD-BRAIN INTERPHASE

Referat (sammandrag)
26T0

Brain vessels are equipped with an enzymatic barrier to neurotransmitter monoamines and their precursors, namely the presence of tyrosine hydroxylase, aromatic aminoacid decarboxylase and monoamine oxidase in the wall (endothelial cells and pericytes) of cerebral microvessels. In larger intracerebral and pial vessels, monoamine oxidase is present both in the wall itself and in perivascular sympathetic nerves; the remaining two enzymes have primarily a neuronal localization. These types of vessels also have catechol-O-methyl transferase in their wall proper. The activity of aromatic aminoacid decarboxylase in cerebral microvessels is high in e.g. rat, dog and man; intermediary in rabbit and cat; and low in baboon. Rat brain microvessels decarboxylate L-DOPA at a maximum rate of approximately 150 ng/g tissue/min.

Circulating neurotransmitter monoamines are primarily prevented from entering the brain already at the luminal membrane of the endothelial lining. The few percent (3-5%) that may pass this membrane are deaminated within the wall of microvessels and, in the case of large parenchymal and pial vessels, in the smooth muscle cell layer, where also O-methylation takes place. By transient disruption of the unity of the endothelial cell lining of the cerebrovascular bed - constituting the morphological blood-brain barrier - through a hypertonic or hypertensive insult, a severalfold increase in the passage of noradrenaline from the circulation into the brain is obtained. The amine is now taken up into the cerebral microvessel wall and also into monoaminergic nerves. Once the barrier is opened, systemically administered noradrenaline induces a pronounced increase in cerebral blood flow - primarily due to a beta-receptor mediated increase in brain neuronal activity - emphasizing the importance of the blood-brain barrier for fundamental cerebral functions.

Referat skrivet av
22T0 Author

Förslag till ytterligare nyckelord
24T0

Klassifikationssystem och -klass(er)
30T0

Indextermer (ange källa)
32T0

Omfång
50T0 140 pp.

Språk
52T0 English

Övriga bibliografiska uppgifter
56T2

Sekretessuppgifter
60T0

ISSN
60T4

ISBN
60T6

Dokumentet kan erhållas från
62T0 Jan Erik Hardebo
Department of Neurology
University Hospital
S-221 85 Lund

Mottagarens uppgifter
62T4

Pris
66T0

The present summary is based on the following publications, which will be referred to by their numerals in the text.

1. Hardebo, J.E., Falck, B., Owman, Ch. and Rosengren, E.: Studies on the enzymatic blood-brain barrier: Quantitative measurements of DOPA decarboxylase in the wall of microvessels as related to the parenchyma in various CNS regions. Acta physiol. scand., accepted for publication. 1978.

2. Hardebo, J.E., Emson, P.C., Falck, B., Owman, Ch. and Rosengren, E.: Quantitative measurement of the formation and degradation of neurotransmitter monoamines at the blood-brain interphase in various species including man. J. Neurochem., submitted for publication. 1978.

3. Hardebo, J.E. and Owman, Ch.: Characterization of the in vitro uptake of monoamines into brain microvessels. Acta physiol. scand., submitted for publication. 1978.

4. Hardebo, J.E., Edvinsson, L., MacKenzie, E.T. and Owman, Ch.: Histofluorescence study on monoamine entry into the brain before and after opening of the blood-brain barrier by various mechanisms. Acta neuropath. (Berl.), accepted for publication. 1978.

5. Hardebo, J.E., Edvinsson, L., MacKenzie E.T. and Owman, Ch.: Regional brain uptake of noradrenaline following mechanical or osmotic opening of the blood-brain barrier. Acta physiol. scand. 101, 342 - 350, 1977.

6. Hardebo, J.E. and Nilsson, B.: Hemodynamic changes in brain caused by local infusion of hyperosmolar solutions, in particular relation to blood-brain barrier opening. Brain Res., submitted for publication. 1978.

7. Edvinsson, L., Hardebo, J.E., MacKenzie, E.T. and Owman, Ch.: Effect of exogenous noradrenaline on local cerebral blood flow after osmotic opening of the blood-brain barrier in the rat. J. Physiol. (London) 274, 149 - 156, 1978.

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CONTENTS

INTRODUCTION

The concept of a barrier between the blood and the brain parenchyma originates from the observations by Ehrlich (1885) that certain aniline dyes pass freely from the circulation into the peripheral tissues but are excluded from the central nervous system. Since then, numerous other substances have been found to be more or less prevented from entering the brain. Studies to clarify the mechanisms underlying the barrier properties have, naturally, included attempts by various techniques to demonstrate a difference between microvessels, constituting the major site of exchange,in the brain and those in peripheral tissues. It soon became clear that such differences do indeed exist. That intracerebral microvessels are unique — precisely in terms of barrier properties — was first demonstrated by Bertler et al. (1964, 1966), who found by histofluorescence and chemical methods that the passage of certain amine precursors, such as the amino acids DOPA* and 5-HTP, into the brain is prevented by an enzymatic mechanism residing in the endothelium and pericytes of the microvessels. Evidence has been presented for several other enzymatic barriers at the blood-brain interphase, such as GABA transaminase (van Gelder 1965) and MAO (Bertler et al. 1966). Shortly afterwards it was shown that central and peripheral vessels in terms of barrier functions differ also in other respects, e.g. in regard to the ultrastructural features of the "sealing" between apposing endothelial cells, paucity of transendothelial pinocytotis, and absence of endothelial fenestrations (Reese and Karnovsky 1967, Westergaard and Brightman 1973) all of which almost totally prevents the passage of macromolecules across the endothelial lining of the brain vascular wall (for review, see Rapoport 1976a).

Normal functions of the brain depend upon an adequate control of the levels of monoamine neurotransmitters and their precursors in the brain extracellular fluid compartment. Accordingly, the entrance of these substances from the circulation

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*Abbreviations used: A: adrenaline, AAD: aromatic L-amino acid decarboxylase, BBB: blood-brain barrier, CBF: cerebral blood flow, COMT: catechol-O-methyltransferase, DA: dopamine, DOPA: 3,4-dihydroxyphenylalanine, 5-HT: 5-hydroxytryptamine, 5-HTP: 5-hydroxytryptophan, MAO: monoamine oxidase, NA: noradrenaline, TOH: tyrosine hydroxylase.

must be strictly regulated, which is accomplished by the BBB. The morphological component of the BBB denotes to a great extent — although not totally (Oldendorf 1971) — the passage of water-soluble and polar substances, like the neurotransmitters, into the brain parenchyma. Decarboxylation of the amine precursor by AAD and deamination of the amine by MAO within the microvessel wall further assist to impede their passage into the brain parenchyma.

AIM OF THE INVESTIGATION

The present study was undertaken in order to elucidate certain properties of the morphological and enzymatic BBB to neurotransmitter monoamines and their precursors. The investigation includes conditions for by-passing these barriers experimentally and how this barrier opening would affect a functional parameter such as the cerebral blood flow. The study was especially focused on the following problems:

(1) The absolute capacity of the brain microvessels to decarboxylate the immediate precursor, and its quantitative relation to the total AAD activity of the brain.

(2) The activity of the various amine-related enzymes at various levels of the brain vascular tree.

(3) Histofluorescence localization of the amine barrier in various parts of the brain vascular tree and in different layers of the vessel wall.

(4) Quantitative estimation of the escape of amines from the brain circulation under conditions with an intact and opened morphological barrier, and histofluorescence localization of the amine leakage beyond the opened barrier.

(5) Since opening of the barrier by hypertonic solutions turned out to be a useful experimental model, a closer study was undertaken of the conditions under which the barrier opening is achieved by these solutions.

(6) A dynamic aspect of the importance of the barrier to vasoactive agents, like neurotransmitter monoamines, was elucidated by studying the effect on the CBF following hypertonic opening of the BBB.

METHODS

Rat and rabbit were the two laboratory animals commonly used in the present study. Principally, the following techniques were used:

In order to study the decarboxylating activity of the BBB, especially in relation to the amine-forming capacity of the brain parenchyma, the formation of DA after administration of its immediate precursor, L-DOPA, was estimated in two different models: after selective blockade of microvascular AAD by administration of carbidopa (Paper 1), and after elimination of central AAD-containing neurons, without affecting microvascular AAD, through spinal cord transection (Paper 1). To obtain a more precise measure of the enzyme activity in brain vessels, a method originally designed for isolation of neurons and glia (for references, see Blomstrand 1971) was further developed so that it became possible to isolate fractions of large and small brain parenchymal vessels with a high degree of purity (Paper 2). Radioenzymatic methods were applied on these fractions to estimate the TOH, AAD, MAO and COMT enzyme activities. Species differences were elucidated by comparing the AAD activities in isolated microvessels from rat, rabbit, cat, dog, pig, cow, baboon, and man (Paper 2).

The cellular uptake of exogenous monoamines at the blood-brain interphase, and the fate of the amines following passage across the endothelial lining when the BBB had been experimentally opened (Paper 3 and 4) was studied by the standard Falck-Hillarp histofluorescence technique (see Björklund et al. 1972). This was correlated with quantitative measurements of the uptake into the brain vessel wall and parenchyma (Oldendorf 1971) of radiolabelled amine (Paper 5). In an attempt to clarify the hemodynamic changes in relation to hypertonic opening of the BBB, urea (and mannitol) was infused into the internal carotid artery under a variety of conditions, and the CBF and intracranial pressure were simultaneously and continuously measured (Paper 6). The cerebral venous outflow method of Nilsson and Siesjö (1976, 1978) was used in the CBF measurements. In order to relate the degree of amine passage across the opened BBB to a more dynamic parameter, quantitative estimation of regional differences in the brain uptake index for circulating NA was performed along with determinations of CBF in the corresponding regions (Paper 5 and 7) using the ^{14}C-ethanol technique (Eklöf et al. 1974).

ENZYMATIC BLOOD-BRAIN BARRIER TO TRANSMITTER MONOAMINE PRECURSORS

Tyrosine hydroxylase activity. The amino acid, tyrosine — the main precursor of the neurotransmittor catecholamines — is present in the circulation at high concentrations (about 10^{-4}M; e.g. Ambrose et al. 1969). The influx to the brain from the

circulation of radioactive trace amounts of L-tyrosine is high (Oldendorf 1971, 1973). This amino acid shares an uptake site for neutral amino acids at the BBB in common with phenylalanine, methionine, histidine, cysteine, valine, isoleucine, leucine, tryptophan, threonine and L-DOPA (Chirigos et al. 1969, Oldendorf 1973, Pardridge 1977). At the blood-brain interphase these neutral amino acids traverse via a sodium-dependent "L-system" (Christensen 1969, Wade and Katzman 1975a), which mediates an equilibrated - not active - bidirectional movement across the cell membranes. Therefore, in the steady state, net uptake rates are considerably lower than influx values, since influx into the brain neurons and incorporation into proteins is balanced by the efflux of amino acids back to the blood as they are released via proteolysis (see also Pardridge and Oldendorf 1977). However, the rate of neurotransmitter synthesis from tyrosine in brain is very low, in the order of pmol/g/min. TOH is present in brain microvessels as well as pial vessels and parenchymal arterioles (Paper 2). In the latter types of vessels the enzyme has primarily a neuronal localization, namely in perivascular sympathetic nerves. It is tempting to postulate that the discrepancy between the high availability of L-tyrosine and the considerable cellular uptake at the blood-brain interphase on one hand, and the regulated central demand of the amino acid on the other, might indicate that the presence of TOH in brain microvessels serves a (bidirectional) barrier function for tyrosine. However, the amino acid carrier mechanism across the blood-brain interphase rather than the enzymatic barrier is probably the main limiting factor in determining the availability of this amino acid to the brain (see Pardridge and Oldendorf, 1977). There is so far no evidence that this presence of TOH reflects extraneuronal synthesis of catecholamine stores; in this respect it rather functions to synthetize catecholamines stored within (non-sympathetic) nerve terminals that are located in close association with the blood vessels and probably to some extent contaminates the microvessel fraction (Paper 2). In this context it is notable that a direct innervation of the endothelial cells and pericytes has recently been demonstrated by electron microscopy (Rennels and Nelson 1975, Swanson et al. 1977).

Aromatic L-amino acid decarboxylase activity. The presence of AAD in the brain microvessel wall, in contrast to larger brain arteries and peripheral vessels, was first shown in the mouse and rat by Bertler et al. (1964, 1966) and Owman and Rosengren (1967). The AAD activity was demonstrated histochemically and chemically after systemic administration of substrates for this enzyme, L-DOPA and L-5-HTP. This finding has later been confirmed in several similar studies (e.g. Bartholini et al. 1971, Dowson 1973), and also by measurement of AAD activity in fractions containing brain microvessels (Lai et al. 1975, Hardebo et al. 1976, Hardebo et al, 1977a, Spector et al. 1977, Paper 2). The circulating L-DOPA concentration is only about 3×10^{-8} M (Hansson et al. 1978), partly due to the efficient peripheral decarboxylation of L-DOPA originating from the gastrointestinal tract. A presence of 5-HTP has still not been detected in the circu-

lation normally (e.g. Magnussen and Engbaek 1978). However, since administration of L-DOPA is of great clinical importance in the medical treatment of Parkinson's disease, and this amino acid (as well as the amine formed) has the advantage of being possible to visualize by histofluorescence techniques, L-DOPA has been chosen as a model substrate when studying AAD activity, even if it may not be the major substrate for microvascular AAD under physiological conditions.

L-DOPA is extracted from the brain circulation to a high extent (Oldendorf 1971, Wade and Katzman 1975b). The uptake into the brain has been characterized as a transport by facilitated diffusion (Wade and Katzman 1975b). The AAD capacity in cerebral microvessels has an upper limit, beyond which unchanged L-DOPA leaks further into the brain parenchyma, as estimated in the rat by determination of the DA formation in two regions of the central nervous system devoid of catecholamine neurons in the parenchyma (cerebellum, and spinal cord caudal to chronic transection) following systemic L-DOPA administration. In these regions about 150 ng DA is formed per g tissue and min (Hardebo et al. 1977b, Paper 1). The upper limit is reached at an intraperitoneal dose of about 100mg/kg (Hardebo et al. 1977b). Considering data presented by Andén et al. (1972) and obtained by ourselves, it can be estimated that this corresponds to a circulating concentration of around 10^{-5} M. The upper limit of AAD activity has also been established by fluorescence histochemistry (Dowson and Laszlo 1971) and by following the fate of radiolabelled L-DOPA (Wade and Katzman 1975b). Decarboxylation in brain microvessels is a rapid process, which has been shown by Wade and Katzman (1975b) following intracarotid injections of trace amounts of L-DOPA: within less than half a minute most of the amino acid injected had been decarboxylated.

Only slight regional differences in the rate of L-DOPA decarboxylation at the level of the BBB have been found: upon intracarotid injection of L-DOPA cortical regions have been shown to have a slightly lower rate than deep hemispherical and brain stem regions (Wade and Katzman 1975b). Further, a slightly lower rate in AAD activity was noticed in the cerebellum as compared to the caudate nucleus and cerebral cortex. This was demonstrated in an in vivo model utilizing a decarboxylase inhibitor (carbidopa) effective only peripherally and in brain microvascular walls (Paper 1) and by determinations on isolated brain microvessels (Paper 2). On the other hand, considerable species differences in microvascular AAD activity exist: the activity in the macaque monkey and baboon is very low, and the enzyme activities in rabbit and cat are also lower than in various other species studied (Langelier et al. 1972, Dowson and Laszlo 1971, Dowson 1973, Paper 2, Hardebo et al. 1979a). A considerable activity is found in man (Paper 2, Hardebo et al. 1979a). Microvascular AAD activity

is noticed already in the fetal brain (Hardebo et al. 1979a). Peripheral microvessels revascularizing a transplant of cerebral tissue, as well as newly formed brain microvessels revascularizing a damaged brain area, are equipped with AAD activity (Björklund et al. 1969, Svendgaard et al. 1975). This is in contrast to the situation when brain microvessels regenerate into a peripheral transplant (Svendgaard et al. 1975) indicating that the barrier characteristics of the newly formed microvessels are determined by the properties of the tissue in which the new circulation occurs.

Also pial vessels and large parenchymal vessels can decarboxylate L-DOPA, but in these vessels the enzyme is almost exclusively located in perivascular sympathetic nerves (Paper 2), whereas the enzyme at the microvascular level is, no doubt, located in the wall itself within endothelial cells and pericytes (Bertler et al. 1966, Paper 2). The active uptake of L-DOPA is very low into the wall of pial vessels (unpublished observation), which can therefore not be considered to form part of a barrier system, in spite of the presence of perivascular adrenergic nerves; on the contrary, already at low circulating concentrations L-DOPA may diffuse through the walls of these vessels to reach the brain parenchyma (Bertler et al. 1966).

The efficiency of the BBB in impeding the passage of L-DOPA into the brain by a decarboxylating mechanism, in addition to the high AAD activity in peripheral tissues (represented by certain visceral organs and the sympathetic nervous system), have required high doses of L-DOPA in the medical treatment of Parkinson's disease with disturbing side-effects as a frequent result. During treatment circulating concentrations of up to about 10^{-5} M have been reported (e.g. Rinne et al. 1973). Partly based on the knowledge about the functions of the enzymatic BBB it has been possible to overcome these problems to a considerable extent: a decarboxylase inhibitor (carbidopa or benserazide), effective in peripheral tissues as well as in brain microvessel walls but only to a negligible extent in the brain parenchyma, is added to L-DOPA in the therapy. Under these conditions, the amine precursor can be administered in lower doses, reducing the incidence of undesired side-effects (as reviewed by Pinder et al. 1976).

Apart from metabolizing circulating L-DOPA - that would otherwise have been taken up across the BBB by the neutral amino acid carrier system - the decarboxylase activity in brain microvessel walls may be involved in a more complex enzymatic barrier mechanism for other circulating amino acids such as phenylalanine, tyrosine and trypto-

phan, or it may function as a link in a hitherto unknown metabolic pathway across the blood-brain interphase.

ENZYMATIC BLOOD-BRAIN BARRIER TO TRANSMITTER MONOAMINES

Monoamine oxidase and catechol-O-methyltransferase. The normal circulating plasma concentrations of neurotransmitter monoamines in man is low: NA about 10^{-9} M (e.g. Meyer et al. 1973), 5-HT in platelet-free plasma about 5×10^{-8} M (e.g. Sommerville 1976 — unless its presence is due to inadvertent rupture of trombocytes). Circulating proteins bind about 50 per cent of the normal plasma concentration of NA, and a smaller fraction of 5-HT (Danon and Sapira 1972). Due to the existence of the morphological BBB, only minor amounts (3 - 5 %) of neurotransmitter monoamines are extracted from the brain circulation, probably uniformly in various regions of the parenchyma (Oldendorf 1971, Paper 5, Hardebo and Nilsson 1979). Indirectly, however, substantial amounts of monoamines may be present in the microvessel walls after uptake of the precursor amino acid followed by local synthesis. A considerable MAO activity is present in the cells (endothelial cells and pericytes) of these brain microvessels (Bertler et al. 1966, Lai et al. 1975, Hardebo et al. 1977, Spector et al. 1977, Lai and Spector 1978, Papers 2, 3, 4).

Prerequisites thus exist for another aspect of the enzymatic barrier mechanism at the level of brain microvessels, namely, degradation of the monoamines by MAO. Further, MAO as well as COMT activity is found in pial vessels (Paper 2), choroid plexus (Lindvall et al. 1979) and parenchymal arterioles (Paper 2; see also Spector et al. 1977, Lai and Spector 1978). Within the wall per se of vessels such as arteries, arterioles and veins, these enzymes are probably located in the smooth muscle cell layer (Verity et al. 1972, Levin and Wilson 1977). In addition, MAO is also present in the perivascular sympathetic nerves. It is usually found that vascular MAO of neuronal origin is primarily of the A type, whereas MAO in the smooth muscle cell layer is primarily of type B (Levin and Wilson 1977). In isolated brain vessels (mixture of large and small vessels) the presence of both MAO A and B has been reported, even after removal of perivascular sympathetic nerves (Lai and Spector 1978). Hence, 5-HT, A and NA (substrates primarily for type A) and DA (substrates to a varying degree for both type A and B) (Houslay et al. 1976, Youdim 1977) may be deaminated within the walls of brain vessels. The primary action of these enzymes in arteries and arterioles may be related to the local inactivation of amines that have a vasomotor function, reaching the smooth muscle media from the perivascular nerves or from the circulation. In this latter respect, the smooth muscle uptake (Gillespie 1973, Buchan et al. 1974) and

breakdown by MAO and COMT of amines that have reached beyond the endothelial lining may also represent a kind of barrier mechanism. This is probably particularly important in the central nervous system, where the passage across the endothelium is low and the subsequent degradation therefore efficient. That a small fraction of NA actually can reach the brain vascular smooth muscle cells and induce a transient vasoconstriction (before the amine is metabolized), is consistent with the finding of a short-lasting reduction in CBF upon intracarotid injection in the rat of a bolus of NA (Hardebo et al. 1979b).

Microvascular MAO, providing an enzymatic breakdown of the amine trapped within the endothelial cells and pericytes, should not only be considered as a gate mechanism for the entrance of circulating neurotransmitters into the brain parenchyma, but also as an inactivation mechanism for excess of neurotransmitter present in the brain extracellular compartment since monoamines are actively taken up into the wall of microvessels from the abluminal side (Hamberger 1967, Paper 3 and 4). The possibility should also be considered that the endothelium with its amine enzymes may not only serve a simple barrier function: the amine uptake (Paper 3) and enzyme mechanisms may play a role in the function of the endothelial cells themselves, which may be target structures for circulating and neurogenically released amines in, e.g., contractile processes (Owman et al. 1977). In this context it should be recalled that a direct innervation of the endothelial cells and pericytes has recently been demonstrated by electron microscopy (Rennels and Nelson 1975, Swanson et al. 1977).

One aspect of the functional role of the presence of MAO and COMT at the blood--brain interphase has been studied in measurements of amine-induced changes in CBF and brain metabolism. The cerebrovascular response to the circulating amines (administered systemically or locally into the internal carotid distribution) can be modified when MAO or COMT is inhibited (MacKenzie et al. 1975, McCalden and Eidelman 1976, McCulloch and Harper 1978, unpublished observations) in a way resembling the effect of amine administered intraventricularly, thus bypassing the BBB (MacKenzie et al. 1975).

The importance of the inactivation mechanism in the microvessel walls can be illustrated by an experiment showing that a transient increase of neurotransmitter monoamine levels occur in the brain extracellular fluid compartment initially during ischemia-anoxia (Meyer et al. 1973, 1974, see also Wurtman and Zervas 1974). This excess — that may be detrimental to the brain through increasing the metabolism in the ischemic-anoxic brain area-may not only be due to an impaired uptake of the transmitter bulk into neurons but also into the microvascular wall locally and into the

choroid plexuses, since all these uptake mechanisms are considerably reduced by anoxia (Tochino and Schanker 1965, Hamberger 1967, Paper 2).

In vitro studies have indicated that human brain MAO is primarily of type B, and that DA in human brain is a type B substrate (Youdim 1977, Glover et al. 1977). Deprenyl, an MAO B inhibitor, has recently been added to the treatment of Parkinson's disease by a combination of L-DOPA and decarboxylase inhibitor (Birkmayer et al. 1977). The therapeutic improvement resulting from this treatment is believed to be caused by an enhanced amount of DA available at DA receptor sites, through inhibition of brain MAO. If the MAO present in human brain microvessels also is of the B type, an additional explanation would be an inhibition of the enzymatic barrier mechanism to DA thus reducing the loss of the amine, both formed in the microvessel wall and in the brain parenchyma from the administered L-DOPA.

EXPERIMENTAL OPENING OF THE BLOOD-BRAIN BARRIER

It can be assumed that a sufficient barrier to monoamine neurotransmitters (DA, NA, A and 5-HT) at the blood-brain interphase is necessary for an adequate neurotransmission function in the brain. Only in the newborn animal, in which the barrier is not yet fully developed, has a substantial uptake of circulating neurotransmitter monoamines been demonstrated (Loizou 1970). The exogenous amines were found to accumulate not only in the brain neurons but also in endothelial cells and pericytes of cerebral microvessels, indicating that microvascular uptake of amines (Paper 3) is working also at this age. In the adult animal, with a fully developed morphological BBB, fluorescence microscopy after systemic administration of DA has indicated that the passage of amines is greatly impeded already at the luminal surface of the brain vessel (Bertler et al. 1966, Paper 4). After systemic injection of NA or DA, amines have been found to leak into the vessel wall only at the level of large pial arteries (Samorajski and Marks 1962, Bertler et al. 1966, Paper 4); these vessels, however, are probably not equipped with a morphological BBB (see discussion by Edvinsson and MacKenzie 1976, Paper 4). Evidence has been presented for a minor passage into the brain parenchyma of circulating 5-HT (Bulat and Supek 1968, Welch et al. 1972), NA and A (Weil-Malherbe et al. 1961, Zakusov et al. 1972). As previously mentioned, the extraction from the brain circulation of trace amounts of NA, A, DA and 5-HT is only in the order of 3 - 5 % (Oldendorf 1971, Paper 5, Hardebo and Nilsson 1979).

Due to the presence of the enzymatic barrier to these monoamines at the blood-brain interphase (see above), it can be assumed that the minor amount of amine leaving the brain circulation is sufficiently trapped already within the walls of the brain vascular tree. The negligible penetration across the intact barrier may be the main reason why no or only little effect on total CBF is seen after intravascular administration (for review, see Edvinsson and MacKenzie 1976).

Various attempts have been made to open the morphological BBB (for review, see Rapoport 1976a). Transient opening by a hypertonic or hypertensive insult have been the most widely applied methods during the last years. A pulse of hydrostatic pressure, elevating the carotid artery pressure above 200 mm Hg, has been shown to open the BBB in the rat as evidenced by extravasation of Evans blue (Rapoport 1976b, Paper 5). Acutely induced extreme systemic hypertension also causes blood-brain barrier damage, probably related to pressure-forced overdistension of the vessels (Johansson et al. 1974, MacKenzie et al. 1976a). The BBB damage by these approaches occurs preferentially in small arterioles and microvessels (Johansson 1974a, Papers 4 and 5). While the barrier damage is primarily confined to vessels in cortical structures of the hemisphere during the systemically induced hypertensive insult, a considerable barrier opening occurs also in deeper structures following a locally induced insult (Rapoport 1976b, Paper 5). A concomitant cerebral vasodilatation aggravates the BBB damage (Johansson 1974b, Johansson and Nilsson 1977). At the ultrastructural level, the barrier damage in systemic acute hypertension comprises a formation of channels in the cytoplasm of endothelial cells, an increased transendothelial pinocytotis and, rarely, opening of tight junctions between the cells (Hansson et al. 1975). It can be assumed that the ultrastructural picture is the same after locally induced pressure increase. Reclosure of the barrier has been shown to occur within half an hour or less (Johansson and Linder 1978, Hardebo 1979). Also intravascular administration of hypertonic solutions of e.g. urea allows large molecules that normally do not penetrate the BBB to pass from the blood into the brain tissue (Rapoport et al. 1972, Brightman et al. 1973, Papers 4 and 7). It was originally suggested that urea extracts intracellular water osmotically from the cerebral endothelial cells resulting in their shrinkage, which was believed to transiently open up the barrier at the tight junctions between contiguous endothelial cells (Brightman et al. 1973). However, hyperosmolar solutions have been shown to cause a considerable, acute rise in the systemic blood pressure (Paper 6), mainly via a central action (Mellander and Hillman 1975), and apparently an impaired ability of the brain vessels to autoregulate (Paper 6). Further, hyperosmolarity has a direct vasodilatory effect (Johnston

and Harper 1973, Wahl et al. 1973, Paper 6). Upon intracarotid infusion of a considerable volume of the hyperosmolar solution also other factors may contribute to a barrier opening, such as increase in intracarotid pressure, increase in blood flow due to hemodilution, and ischemia (Paper 6). That opening of BBB by a hypertonic solution is not only due to osmotic shrinkage of the endothelial cells (in the concentrations normally used to open the barrier) is also emphasized by the fact that blocking the increase in systemic blood pressure will minimize the barrier damage (Hardebo 1979). Furthermore, no opened tight junctions have actually been demonstrated ultrastructurally, but instead an increased transendothelial pinocytotis (Hansson et al. 1979), resembling findings during barrier opening induced by an acute hypertensive insult. The hypertonic barrier opening is reversible within a few hours (Rapoport 1972, Pollay 1975, Chiueh et al. 1978, Paper 5, Hardebo 1979).

Following a hypertonic or hypertensive insult, the brain uptake for various substances, including NA, in regions where the barrier has been opened, can be enhanced severalfold (Pollay 1975, Johansson 1976, Chiueh et al. 1978, Rapoport et al. 1978, Paper 5, Hardebo 1979). When the blood-brain barrier is opened experimentally, an accumulation of monoamines in the cells of the microvessel wall is clearly distinguishable by fluorescence microscopy (Flodmark et al. 1969, Paper 4). Under these conditions the monoamines may enter the cytoplasm of the endothelial cells by pinocytotis (Hansson et al. 1975), or it may pass between (or through) the endothelial cells to reach the abluminal side of the endothelial membrane and from here enter the endothelial cell as well as the pericyte. An active uptake of monoamines into the microvessel walls is exclusively working across the abluminal membrane of the endothelial cell (Paper 3), in direction from the brain into the cytoplasm of this cell (and of the pericyte). This would explain why monoamines, also when they are administered intraparenchymally (Bertler et al. 1966) or intraventricularly (Fuxe and Ungerstedt 1968), accumulate in the microvessel walls in a narrow zone around the stich channel and periventricularly, respectively.

In contrast to the weak effect of neurotransmitter amines on the CBF and brain metabolism when injected into the circulation of an animal with intact BBB (see Edvinsson and McKenzie 1976), the same substances and concentrations induce substantial changes in these two parameters following barrier opening by a hypertonic or hypertensive insult (MacKenzie et al. 1976b, Harper and MacKenzie 1977, Paper 7), or when the barrier is circumvented by intraventricular administration of the amine (MacKenzie et al. 1976b).

When studying the degree of penetration of circulating amines of high biological activity, the question arises whether the amine itself may influence the permeability of the BBB. In fact, there is some experimental evidence that a high concentration of e.g. NA or 5-HT (Westergaard 1975a, b) may induce an enhanced vesicular transport across the cerebrovascular endothelial cells. However, it is highly unlikely that this factor influences quantitative measurements of the brain uptake index, in which only trace amounts of the amine are used.

CONCLUSIONS

Brain vessels are equipped with an enzymatic barrier to neurotransmitter monoamines and their precursors:

(1) Brain microvessels (capillaries and venules) were in rat found to decarboxylate L-DOPA at a maximum rate of approximately 150 ng/g tissue/min. The microvascular catecholamine forming capacity represents 25% of the total capacity in a region rich in catecholamine nerve terminals (caudate nucleus) and as much as 70-90% in regions poor in such nerves (cerebellum and spinal cord caudal to a transection).

(2) The activity of aromatic L-aminoacid decarboxylase in cerebral microvessels is high in rat, dog, pig, cow and man; intermediary in rabbit and cat; and low in baboon. In addition to this enzyme, cerebral microvessels also contain tyrosine hydroxylase and monoamine oxidase. In larger intracerebral and pial vessels monoamine oxidase is present both in the wall itself and in perivascular sympathetic nerves; the remaining two enzymes have primarily a neuronal localization. The latter types of vessels also have catechol-O--methyltransferase in their wall proper. The microvessel fraction studied is probably contaminated with perivascular nerves which represent at least part of the tyrosine hydroxylase activity in the fraction, but an enzymatic barrier function at the blood-brain interphase is also possible.

(3) Circulating neurotransmitter monoamines are primarily prevented from entering the brain already at the luminal membrane of the endothelial lining. The few percent that may pass this membrane are deaminated within the endothelial cells and pericytes of brain microvessels and, in the case of large parenchymal and pial vessels, in the smooth muscle cell layer, where also O-methylation takes place.

The unity of the endothelial cell lining of the cerebrovascular bed constitutes a morphological blood-brain barrier mechanism to neurotransmitter monoamines:

(4) The brain uptake index for noradrenaline is in the rat about 4% without any overt regional difference. Transient opening of the morphological barrier by hypertonic or hypertensive insult causes a several fold increase in the passage of noradrenaline from the circulation into the brain, where it enters the wall of cerebral microvessels and is also taken up by monoaminergic nerves.

(5) The barrier opening by hypertonic solution is, a least partly, accomplished by increase in systemic pressure and cerebral vasodilatation, which will induce changes

in the endothelial cell lining that allows for the passage of substances not normally passing the barrier to any great extent.

(6) Once the barrier is opened systemically administered noradrenaline induces a pronounced increase in cerebral blood flow, primarily due to a beta-receptor mediated increase in cerebral activity, emphasizing the importance of the blood-brain barrier for fundamental cerebral functions.

ACKNOWLEDGEMENTS

It has been a priviledge for me to work and co-operate within the monoamine research group at the Department of Histology, University of Lund. I wish to express my deep gratitude to:

Professor Christer Owman, my teacher, co-worker and friend, under whose excellent guidance it has been a pleasure to work.

Professor Ragnar Müller, for arranging excellent working conditions, for taking a personal interest in my research work and constantly supporting it.

Professor Bengt Falck, for the kindness to give the opportunity to perform this work at his department and for stimulating cooperation.

I wish to thank Drs. Sture Axelsson, Lars Edvinsson, Eric MacKenzie, Bengt Nilsson and Evald Rosengren for stimulating collaboration and valuable discussions.

I have received invaluable technical assistance from Ulla-Britt Andersson, Camilla Hultman, Inga-Margit Hägg, Gertrud Stridsberg and Mary-Ann Sällström. I am also indebted to Siv Carlson, Bodil Stache, Agneta Valler for skilful typing of the various manuscripts.

Anne Charlotte, thank you for patience.

This work was supported by the Swedish Medical Research Council (Grants No. 04X-732 and 04X-56), the Faculty of Medicine, University of Lund, Sweden, the European Brain and Behaviour Training Programme and the Wellcome Trust.

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